Vol. XVII. *July, 1909.* *No. 2.*

BIOLOGICAL BULLETIN

KARYOKINETIC FIGURES OF CENTRIFUGED EGGS; AN EXPERIMENTAL TEST OF THE CENTER OF FORCE HYPOTHESIS.

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In the annelid *Chætopterus* the first maturation spindle forms in the unfertilized eggs after they are shed in the sea-water, and develops to the stage of the mesophase (Fig. 1). Here it remains standing without change, it may be for hours, unless the egg be fertilized or otherwise effectively stimulated. The eggs can be obtained at Woods Hole in unlimited amounts for a period of about two months during the summer, and as they may be taken from the worms at any time of day they furnish ideal material. The possibility of experimenting directly on a definite stage of the karyokinetic figure is naturally suggested by the material itself, and I propose to give here an account of the results of experiments with centrifugal force, which have been carried on during four successive seasons, so far as they are related to the karyokinetic figure. A communication of the results was made before the joint session of the Central Branch of the American Society of Zoölogists and Section F (Zoölogy) of the American Association for the Advancement of Science held in Chicago, December, 1907, and an abstract was printed in *Science*, N. S., Volume XXVII., pp. 907–908, June 12, 1908.

The principal advantage of this material, aside from its availability and abundance, is that it offers the rare opportunity of experimenting on a *definite* and *fixed* stage of the karyokinetic figure. At the stage in question the forces concerned in karyokinesis have reached a certain balance which is maintained indefinitely, until the equilibrium is upset by the conditions initiated by entrance of the spermatozoön or some other effective change.

The action of potassium chloride appears to be quite specific in this respect, as was first discovered by Mead ('98), and since described by Loeb ('01) and myself ('02). Such agents set in motion the series of karyokinetic processes, which are then carried through to their completion. But even violent mechanical disturbances, such as are produced by shaking or centrifuging, cause only temporary disturbances, and the equilibrium of the mesophase of karyokinesis is soon reëstablished. The methods

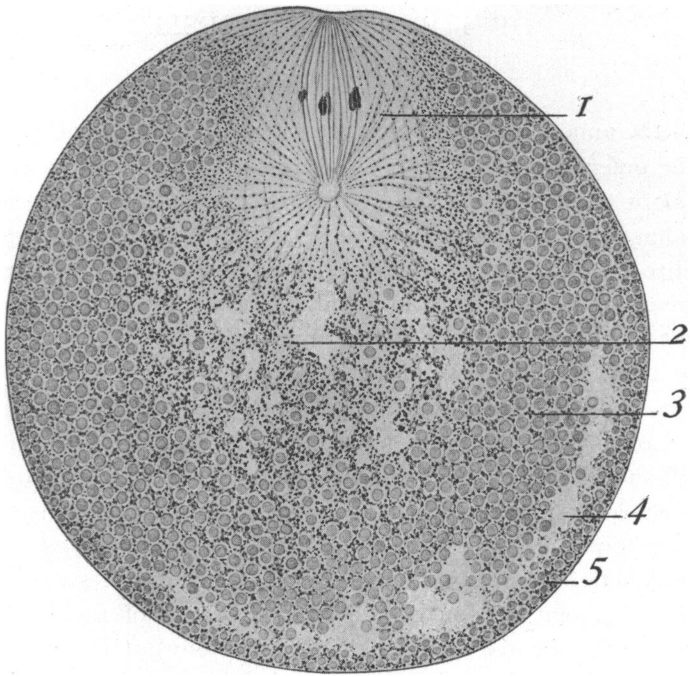

FIG. 1. Section of an egg of *Chætopterus* at the stage used for the experiments. See text for description. 1, karyokinetic figure; 2, central spongy area; 3, inner dense zone; 4, outer spongy area; 5, ectoplasmic layer or outer dense zone.

by which the regulation, or regeneration, of the karyokinetic figure is brought about after centrifuging appear to me to have some bearing on the problem of the nature of the dynamical complex represented by the karyokinetic figure.

Fig. 1 shows the typical condition of the eggs before centrifuging. The animal hemisphere contains a large karyokinetic figure at the mesophase with nine chromosomes in the equatorial

plane. The outer pole of the spindle is practically in contact with the surface of the egg, which is slightly depressed at the pole. The centrosome of the outer pole is smaller than that of the inner pole. The astral rays cross very extensively opposite the equatorial plane; indeed, rays from the outer pole may extend inward beyond the level of the inner pole, and rays of the latter may extend nearly to the surface of the egg. In the spindle itself, there is the usual distinction between mantle fibers attaching the chromosomes to the two poles and central spindle fibers running from pole to pole. The interfibrillar spaces are perfectly clear. The only asymmetrical feature of the spindle is the somewhat smaller size of the outer pole and the difference in arrangement of the rays at the two poles, owing to the proximity of the outer pole to the surface of the egg.

All eggs show great uniformity, but there may be slight differences between eggs of the same lot in the following respects: (1) In some the outer pole is not actually attached to the surface so as to produce a depression, but may be removed from the surface by a distance of about twice the diameter of the outer centrosome. There is thus a variation in the firmness of fixation of the spindle at the animal pole. (2) There is some variation in the intensity, that is, in the length and strength of the astral rays, which appear less well developed in some cases than in others. In all cases, they cross to a considerable extent in the equatorial plane.

The evidence that is to be presented is furnished by the results of centrifuging the eggs at varying speeds and for various times. To form a proper conception of what happens it is absolutely essential to understand the effects of centrifugal force on the protoplasm. In a previous paper I presented an analysis of this subject and showed that the fundamental structure of the egg as represented by the conformation of the ground substance is not essentially modified by the centrifugal force; it is at most somewhat distorted. On the other hand, the granules suspended in the ground substance move through the latter in a central or distal direction, depending on their relative specific gravities, and arrange themselves in three strata: the stratum nearest the axis of the centrifuge is of course of least specific gravity; it consti-

tutes a cap of granules of intermediate size that give a fatty reaction with osmic acid and are soluble in oils. The intermediate stratum, forming a *hyaline band*, contains very minute granules that take the basic stain ; they may therefore be called basophile granules. The distal stratum occupies the distal hemisphere of the egg and is composed of larger granules of yellow tinge, usually called yolk granules, which take the acid stain and may be known collectively as acidophile granules. The chromatin and the spindle have the same specific gravity as the basophile granules, as is proved by the fact that they are found in the hyaline band when they move freely.

The movements of the granules, and consequently their segregation, are modified by differences of resistance within the ground substance. The latter has four concentric zones (Fig. 3, *A*), (1) a central more fluid area (Fig. 3, *A*, 2), (2) a dense zone bounding the latter (Fig. 3, *A*, 3), (3) a second more fluid zone specially developed in the region of the spindle (Fig. 3, *A*, 4), and (4) an ectoplasmic zone defective at the outer end of the spindles (Fig. 3, *A*, 5) (see Lillie '09).

All zones contain both basophile and acidophile granules (Fig. 1). Under the action of centrifugal force, therefore, the granules move relatively freely through the more fluid zones and are impeded in their movements in the denser zones. The consequence is that when low centrifugal speeds of short duration are used, there is a tendency for the granules to aggregate in the dense zones. Thus the basophile granules of the central more fluid area aggregate in the central end of the dense zone bounding it (Fig. 2) and the acidophile granules in the distal end. The basophile granules of the outer fluid zone aggregate proximally with reference to the axis of the centrifuge and the acidophile granules aggregate distally, and therefore lie external to the inner dense zone and within its walls, but do not pass through the latter to enter the central more fluid area (Fig. 2). The most important of these aggregations with reference to the interpretation of the karyokinetic figure is the *basophile cap* found in the central end of the inner dense zone (Fig. 2, *b.c*).

The distortion of the ground substance is of the following character: (1) the egg is invariably somewhat elongated in the

line of action of the centrifugal force, owing to the differential force between centripetally and centrifugally moving granules; (2) the principal part of this elongation is born by the massive, external, more fluid zone, which is stretched more on the centrip-

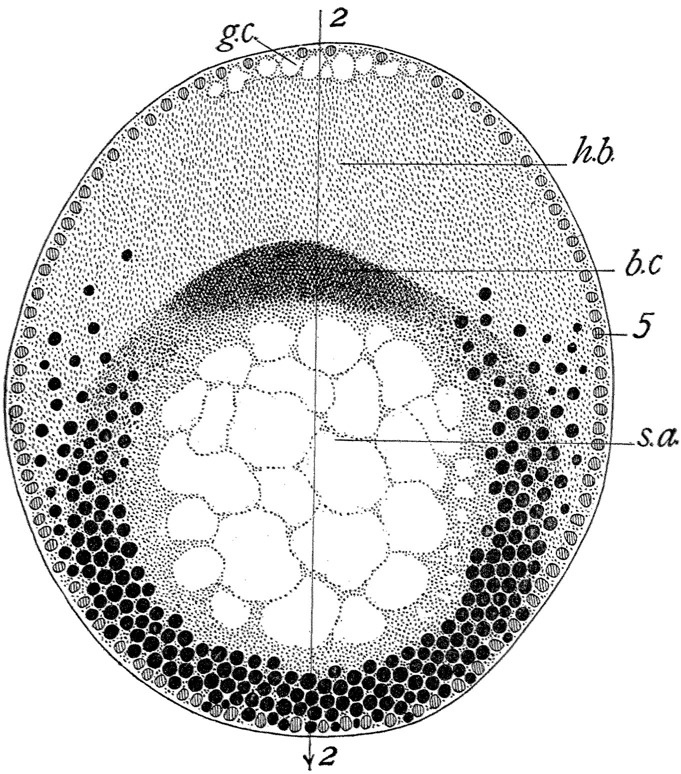

FIG. 2. Section of an egg of *Chætopterus* centrifuged 1,150 revolutions in 31 seconds at a radius of 13 cm. 2–2 indicates the direction of the centrifugal force; 5, ectoplasmic zone; *b.c.*, basophile cap; *g.c.*, gray cap, or fatty granules (dissolved out by the oil used in preparation); *h.b.*, hyaline band; *s.a.*, spongy area. This egg was stained in iron hæmatoxylin and orange G.

etal side, the consequence being that the inner fluid area and its dense bounding zone lie on the centrifugal side of the middle point of the elongated egg (Figs. 3 and 4).

The spindle itself may be moved as a result of centrifuging. The simplest cases are those in which the outer end remains fixed and the inner end is swung from its radial position. This shifting appears to be due to distortion of the ground substance with

the result that the inner dense zone presses in one or another direction on the spindle and displaces its inner end. In other cases the spindle is moved some distance from the surface into the hyaline band; this result is more commonly found with the higher centrifugal powers.

The normal relation of the spindle to the zones of the ground substance is illustrated in Figs. 3, *A*, and 4, *A*. The inner end of the spindle reaches to the inner dense zone and its antipolar rays are embedded in the latter, which is indented by the spindle. If now the centrifugal force acts parallel to the axis of the egg with the animal pole in a central position, the egg elongates in this axis. If the outer end of the spindle remains attached to the surface, the inner end tends to be withdrawn from the inner dense zone, and the basophile cap formed lies outside of the area of

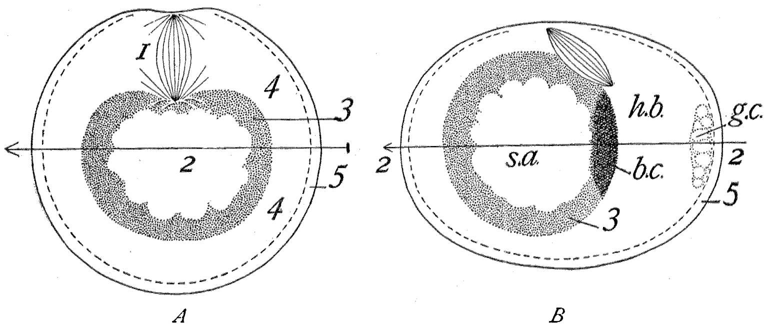

FIG. 3. *A*, diagram of zones of the ground substance in the normal egg and the relations of the spindle. *B*, diagram to illustrate the effect of centrifugal force on the ground substance and spindle acting in the direction of the arrow. 1, karyokinetic figure; 2, central spongy area of control egg; 2–2, direction of action of centrifugal force; 3, inner dense zone; 4, outer more fluid zone; 5, ectoplasmic zone. The numbers have essentially the same significance as in Fig. 1; *b.c.*, basophile cap; *g.c.*, gray cap; *h.b.*, hyaline band.

the spindle. If, however, the inner end remains fixed, the outer end is withdrawn from the surface, and the inner end is embedded in the basophile cap.

If the centrifugal force acts at right angles to the axis of the egg, the inner end of the spindle tends to be swung in a proximal direction, (Figs. 3, *A* and *B*) both because the specific gravity of the spindle as a whole carries it in that direction, and also because the contraction of the egg in its own axis brings the dense zone

nearer the animal pole and thus exerts pressure on the spindle. Many cases of this kind are found.

If the animal pole is distal in position in the centrifuge the spindle must be subjected to rather violent assaults: first, because its specific gravity tends to drive it towards the opposite end of the egg; second, because the basophile granules are streaming away from it and the acidophile granules rushing in, and third, because the inner dense zone is approximated to this pole (Fig. 4, *B*). I have not found any eggs with the spindle fixed at the distal pole after centrifuging, and am therefore forced to assume that in all cases in which the centrifugal force acts in this direction the spindle is torn loose from the surface and moves up into the hyaline band.

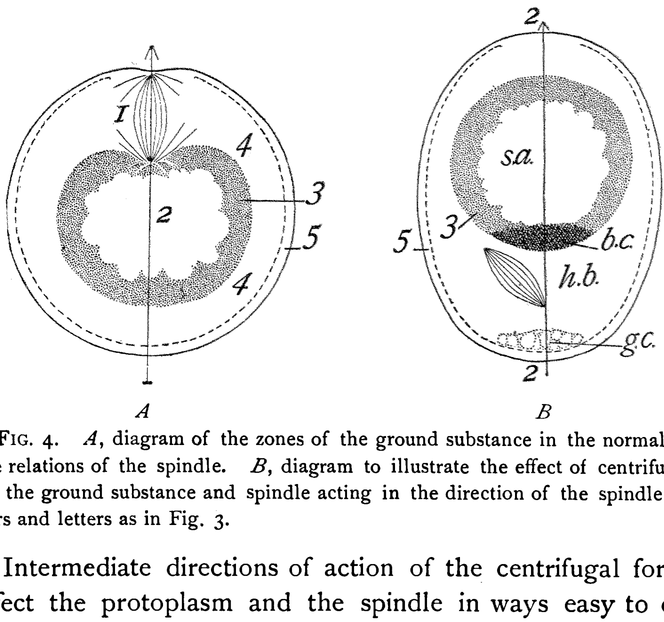

FIG. 4. *A*, diagram of the zones of the ground substance in the normal egg and the relations of the spindle. *B*, diagram to illustrate the effect of centrifugal force on the ground substance and spindle acting in the direction of the spindle. Numbers and letters as in Fig. 3.

Intermediate directions of action of the centrifugal force will affect the protoplasm and the spindle in ways easy to deduce from the above.

If the above analysis of the effects of centrifugal force is correct, or even partially so, we have to distinguish between those cases in which granules are driven out of, or into, the area of the karyokinetic figure, and those cases in which the spindle has moved its position. It is obvious that both conditions may occur simultaneously; indeed, it is probable that this is almost always the case.

In my paper of 1906, I described and illustrated a naked spindle with centrosomes at its ends, but practically no radiations. This spindle was found in an egg that had been centrifuged about 2,000 revolutions in a minute at a radius of 13 cm., killed in picro-sulphuric acid, sectioned, and stained in Delafield's hæmatoxylin. At the time, I was not paying special attention to the question of analysis of the karyokinetic figure, and I regarded the case as typical.

When I repeated the experiments in the summer of 1906, with special reference to the analysis of the karyokinetic figure, I soon found that the condition of a naked spindle after centrifuging was not typical; on the contrary, it is extremely rare and exceptional. Well-developed asters are the rule after centrifuging, though they may exhibit certain asymmetries or other modifications of the normal condition. Either, then, the rays must persist in spite of movements of the spindle and passage of granules through them, or the naked spindle must have formed new radiations.

Presumably the latter process would require time, and it therefore seemed possible that if special pains were taken to fix the eggs in the shortest possible time after centrifuging, one might find at least a considerable proportion of naked spindles. But this also proved a vain expectation. Typically, astral radiations are well developed after centrifuging; and this is the case no matter how quickly the eggs are killed, nor whether the speed be high or low, within the limits of the actual experiments.

I therefore considered the possibility of fixing the eggs during the action of the centrifugal force, but I did not see clearly how to compass this end and so the experiment was never performed.

I. Movements of the Spindle.

Every case in which it can be demonstrated that the spindle has moved through the cytoplasm furnishes a test between mitome and centrosome hypotheses. If the visible rays are organic radii attached to the poles of the spindle (mitome hypothesis), they must be disarranged by each movement of the latter, and movements of the spindle at different angles to its own axis must produce different characteristic modifications of the rays of

the aster. If the spindle moves in the direction of its own axis, for instance, the rays of the forward aster must tend to be bent back about the spindle, and those of the other aster away from the axis. Similarly, there should be definite configurations characteristic of every angle and amplitude of movement. But, if the rays are expressions of forces centered at the poles and are therefore composed of oriented particles (centrosome hypothesis), such disarrangement of the radii would not be expected to result from movements of the spindle, except to the extent involved in the inertia of the substances concerned.

In a considerable number of experiments it was my aim to kill the eggs in the shortest possible time after centrifuging. The tube was removed from the centrifuge as quickly as possible, the water poured off and the killing fluid poured on immediately. Therefore, in two or three seconds after the centrifugal force had ceased to act the eggs were submitted to the action of the killing fluid. Such eggs were sectioned and stained.

The cases that interest us most here are those in which the spindle is found detached from the surface, because it is obvious that such spindles have been moved from their original position: The entire karyokinetic figure in such cases may be perfectly symmetrical if it is found in protoplasm of uniform composition. But differences in the composition of the protoplasm within the area of the karyokinetic figure are correlated with asymmetries of the figure itself which are considered beyond. If the outer pole of the spindle is withdrawn from the surface it is found to possess well-developed antipolar rays, although these were entirely wanting before centrifuging (cf. Fig. 5), and the astral rays are symmetrical around both poles, although "organic radii" would naturally be distorted by such displacement of the "centers of insertion" as is illustrated in Fig. 5. It appears to me that the astral radiations in such cases must be regarded as new. The case is of course analogous to the shifting of sperm asters in fertilization. In the present case, however, we know the time of readjustment to be very short, one minute at the outside, and the initial and reconstructed conditions are before us for comparison (cf. Figs. 1 and 5).

Similar cases are numerous in eggs that have been submitted

to a very strong centrifugal force, 7,500 revolutions in one minute at a radius of 6 cm., because the end effect of such centrifuging is to produce a uniform arrangement of basophile granules in the hyaline band, into which the spindle also is driven. We there-

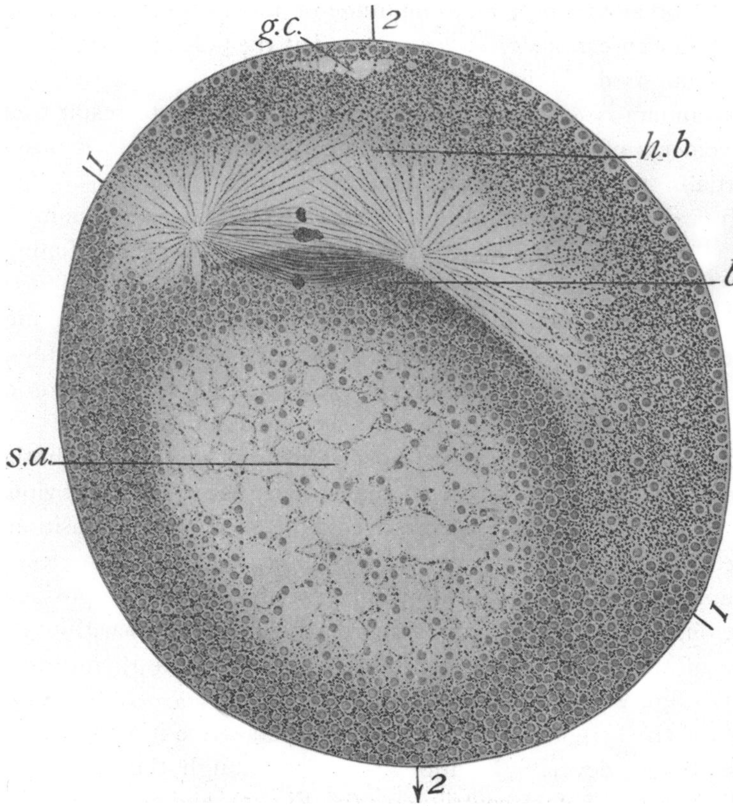

FIG. 5. Section of an egg of *Chætopterus* centrifuged 1,150 revolutions in 31 seconds at a radius of 13 cm. 1–1, primary axis of the egg; 2–2, direction of action of the centrifugal force, secondary axis; *b.c.*, basophile cap; *g.c.*, gray cap; *h.b.*, hyaline band; *s.a.*, spongy area. The egg was stained in thionin and orange G.

fore never find such particomposition spindles as in low powers of the centrifuge (see beyond). The karyokinetic figure is usually symmetrically developed after the strongest centrifuging in spite of the fact that rearrangements of granules are more extensive than with lower centrifugal powers. The reason for this must lie in the uniform character of the protoplasm, which is produced in the hyaline band by strong centrifuging.

A single example of this kind would not be convincing in itself, for it might be maintained that the egg in question deviated originally from the norm of the control eggs. But when such a

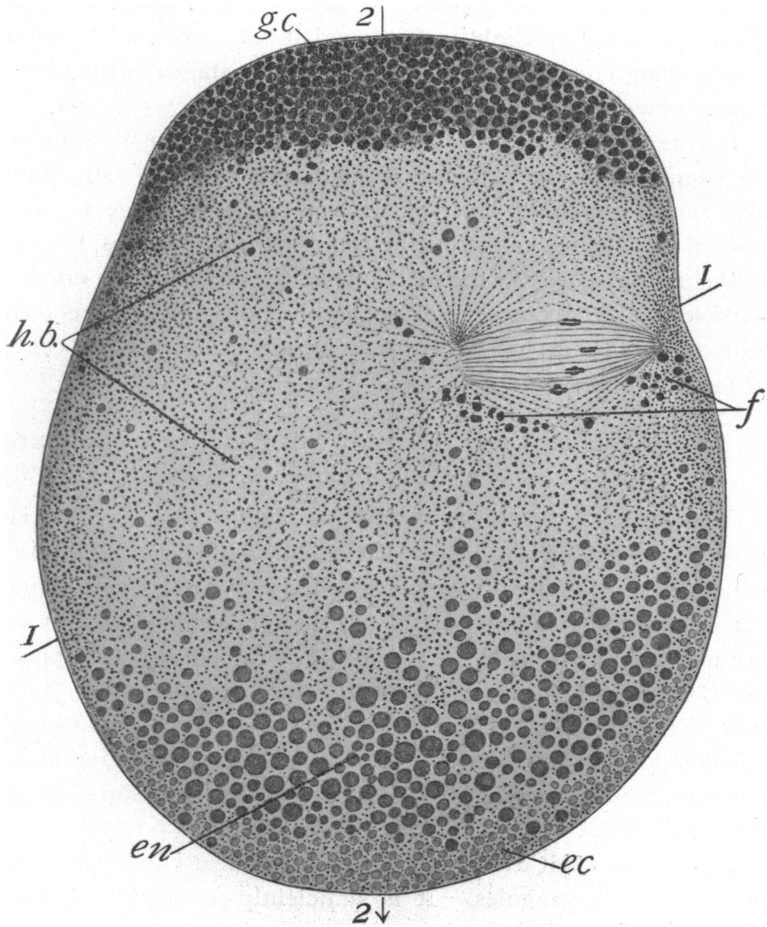

FIG. 6. Section of an egg of *Chætopterus* centrifuged 7,800 revolutions in one minute at a radius of 6 cm. The egg was killed in Flemming's fluid, and the fat granules of the gray cap are stained black. The spindle blocks fat granules passing in a centripetal direction (*f*). 1–1, primary axis of the egg; 2–2, secondary axis; *ec.*, ectoplasmic spherules; *en.*, endoplasmic spherules; *f.*, fat granules blocked by the spindle; *g.c.*, gray cap; *h.b.*, hyaline band.

condition is found to be the type of hundreds of eggs in different experiments with no considerable deviations, as is the case in my

experiments, it becomes a conclusive argument for the re-formation of rays in successive positions of the spindle, a condition that can be explained only on the centrosome hypothesis.

Among the numerous cases we find some in which the antipolar rays are very slightly developed at *both* ends; and various intergrading conditions that seem to indicate stages in the formation of new rays.

In the case of eggs submitted to low centrifugal force an occasional karyokinetic figure is found with distorted astral rays. But such a condition must occur as a transition stage in every case of spindle movement on the centrosome hypothesis, because of the mere viscosity of the medium and the inertia of the oriented particles. The occurrence of such conditions is therefore to be expected, and does not furnish any argument against the center of force hypothesis.

2. Movements of Granules into the Area of the Karyokinetic Figure.

The three classes of granules, basophile, acidophile, and fat, may be moved into the area of the karyokinetic figure. As a general proposition we may say that fatty or acidophile granules driven into the area of an aster tend to destroy the radiations and are not themselves arranged in lines. If forced into the spindle itself, they tend to disarrange its fibers. On the other hand, basophile granules that are driven into the area of the asters or spindle do not, apparently, destroy the existing rays or fibers, and are themselves arranged conformably to the lines of the figure.

A. We need not dwell long on the statement concerning fatty and acidophile granules. It is sufficiently supported by Figs. 5 and 6 as regards the asters. No traces of rays are left in invaded areas of the asters except that the scanty basophile granules between the fatty or acidophile granules may show a slight radiate tendency centered at the poles of the spindle. There are no detached rays, no traces of broken down threads; the peripheral ends of rays of invaded areas always disappear absolutely.

Serious disarrangement of the fibers of the spindle is found if fatty or acidophile granules are driven into it. Such cases are

relatively rare, but the disarrangement may be much more considerable than in the case illustrated (Fig. 7). The ends of the spindle may be much spread out and the chromosomes may be turned around at right angles to their normal orientation.

B. The effect of the introduction of basophile granules into the area of the karyokinetic figure is, however, entirely different. They cause no disarrangement, but appear to become oriented like the rays of the aster, or the fibers of the spindle. The evi-

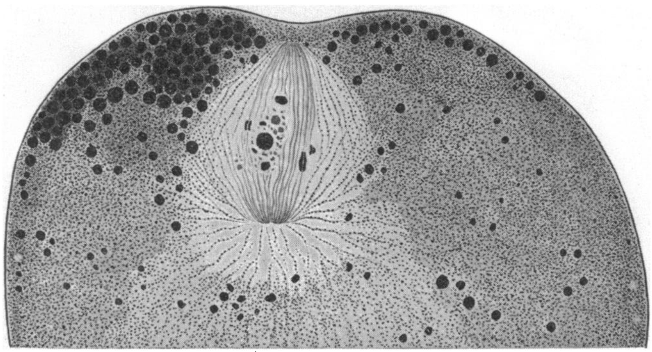

FIG. 7. Section of an egg of *Chætopterus* centrifuged 2,300 revolutions in 70 seconds at a radius of 13 cm. Killed in Flemming's fluid. The figure represents the centripetal half of the egg. Fat granules have entered the spindle and destroyed part of its structure.

dence for this conclusion is based on the behavior of the dense aggregation of the basophile granules found in the central end of the inner dense zone after the action of low centrifugal powers, *i. e.*, of the basophile cap (Figs. 2, 3*B*, and 4*B*).

The heavy stain of the basophile cap lies entirely in the granules, not at all in the ground substance. It is true that the ground substance between the granules appears darker in the basophile cap than elsewhere, but this is an effect due to the light passing through basophile granules at a lower focus. In thin sections it is not evident at all. The conclusion is really proved by the fact that such an effect is seen only in the basophile cap, and that this may lie in any part of the wall of the dense zone, depending on the direction of action of the centrifugal force.

1. Let us note first that the granules that compose the basophile cap have a uniform distribution when remote from the

spindle (Fig. 2). What happens when they are driven into the area of the karyokinetic figure? A few examples will illustrate:

The egg shown in Fig. 8 was allowed to stand in sea-water about 35 minutes, then centrifuged 1,150 revolutions in 31 seconds at a radius of 13 cm. and killed as rapidly as possible in picro-acetic acid. It was one of a large number. Sections were cut 6 μ thick and stained in iron hæmatoxylin and orange G.

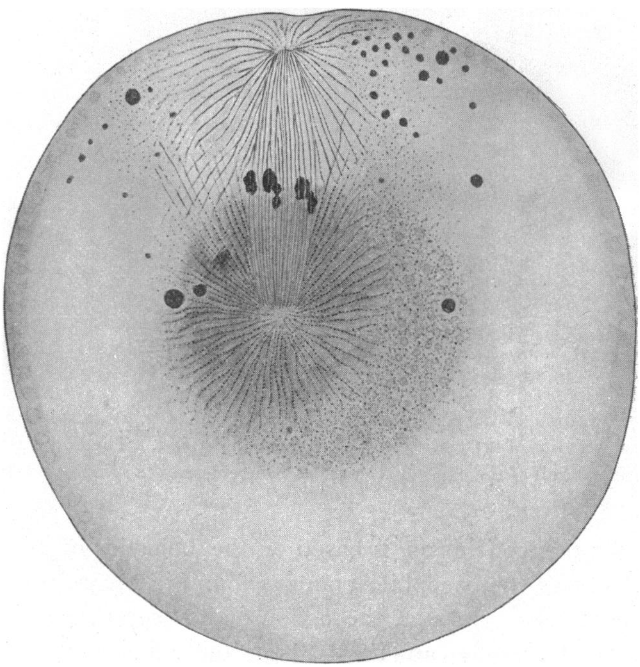

FIG. 8. Section of an egg of *Chætopterus* centrifuged 1,150 revolutions in 31 seconds at a radius of 13 cm. Half of the karyokinetic figure lies in the basophile cap. See text for further description.

All the sections of this egg are present on the slide. The figure represents a single section which includes practically the entire spindle and all nine chromosomes. The plane of the section was nearly transverse to the axis of stratification and is approximately tangential to the basophile cap; but on the right side the section passes nearer to the distal pole than on the left side and therefore takes in a good deal of the distal acidophile stratum on this side, while on the left side the section is confined to the

hyaline band. The granules of the ectoplasmic layer are apparently unaffected by the centrifugal force. The karyokinetic figure has been moved little, if at all, as is proved by the fact that there is an indentation opposite the outer pole, and by the relation of the ectoplasmic layer which exhibits the characteristic defect around the outer pole of the spindle, which I described in my first paper.

That portion of the karyokinetic figure lying within the basophile cap partakes of its character, and is as sharply differentiated from the remainder of the figure as the basophile cap itself is differentiated from the protoplasm of the hyaline band. As to the nature of these differences: (1) the stain is much darker within the basophile cap; (2) the rays of the aster are more

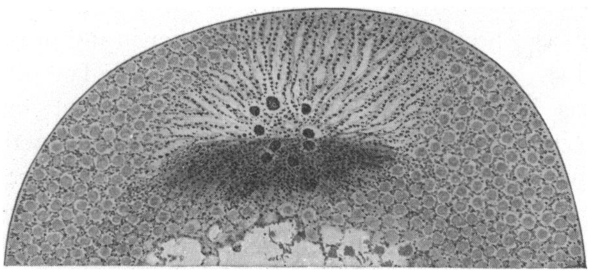

FIG. 9. Transverse section of parti-composition spindle. From section of an egg of *Chætopterus* centrifuged 1,150 revolutions in 31 seconds at a radius of 13 cm. Half of the section of the spindle lies in the basophile cap. See text for further description.

numerous than in the control (Fig. 1) and also more granular; they are in fact obviously made up of linear arrangements of basophile granules; (3) the spindle itself is more darkly stained within the basophile cap than outside of it; this pertains to the fibers themselves, but I would not venture to say that the fibers are more numerous.

Where a fiber either of the spindle or of the aster crosses the boundary between the basophile cap and the hyaline zone its character changes. Rays of the aster never extend into a compact mass of acidophile granules, hence the present figure is less developed on the right side than on the left.

Fig. 5 represents a spindle placed tangentially to the basophile cap. The contour of the spindle is perfectly regular, but its

composition is heterogeneous. For the rest, exactly the same principles apply as in Fig. 8, except that astral rays are not very numerous in the basophile cap; this is due to the fact that the section is near the margin of the cap and contains therefore a large proportion of acidophile granules.

Fig. 9 finally is a transverse section across a spindle lying partly in and partly out of a very dense basophile cap. It is obvious that the spindle area has been invaded on one side by the basophile granules. These are in fact so numerous and are stained so deeply that they almost conceal the chromosomes embedded in them. The concentration of the granules within the spindle area is thus extremely different on the two sides, but on each side it is precisely the same as in the neighboring cytoplasm.

These cases are typical of a large number in my preparations. It is obvious that there is a great difference in the fatty and acidophile granules on the one hand and the basophile granules on the other, with reference to their effect on the karyokinetic figure. The former efface any part of the figure which they occupy, the latter are arranged conformably to the lines of the figure. This difference is not conceivably a mere question of size, it is rather a question of specific behavior. The basophile granules behave as though they were within effective range of centers of force to which they are permeable; the fatty and acidophile granules on the other hand behave as impermeable particles would behave in such a field.

It is worth while to examine this idea more carefully because the center of force hypothesis alone can render account of the results of these experiments. Theoretically, on a center of force hypothesis the following results might be expected: (1) That the number of radiations from the center would be a factor of the density of aggregation of the more permeable particles; if the number of permeable particles within the effective area were greatly increased the number of rays should therefore become greater. (2) The number of oriented particles in the same length of any ray should also be a factor of the density of aggregation of the permeable particles.

We would therefore expect on the center of force hypothesis

of the karyokinetic figure that, if the basophile granules are permeable to the force, (1) they would be oriented along the lines of force when driven into the area of the karyokinetic figure; (2) that the number of rays in an unusually dense aggregation of basophile particles would be greater than usual and (3) that the number of oriented particles in each ray in such cases would be greater than usual.

These appear to be the conditions found in my experiments. With reference to the first condition, there can be no question that the basophile granules arrange themselves conformably to the lines of the karyokinetic figure, as already noted. With reference to the number of astral rays one obtains a strong subjective impression that they are more numerous in the basophile cap than in the asters of control eggs, and I have attempted to confirm this by actual counts. To do this it is necessary to divide the aster into sectors for comparison, for the reason that asters lying entirely within the basophile cap do not occur, owing to the form of the basophile cap. I have therefore made comparisons between sectors of 90° of the normal aster and asters of the basophile cap. One has further to restrict the count by limiting it to a single focus of the microscope. Under these conditions I found an average of 8 rays to the 90° sector of the normal aster (eighteen counts) and 12.5 in 90° sectors of asters in the basophile cap (six counts). The difference seems too great to be accounted for by error. The counts confirm the impression that one receives by mere comparison. I do not think that such increase of the number of rays in a sector of the aster could be explained by compression of preëxisting rays from other sectors of the same aster, for they are quite uniformly spaced and are as straight and regular as the normal rays.

As regards the third condition: rays within the basophile cap stain more strongly than those without; but as the stain is held by the granules, this would be evidence for a greater number of granules in the rays. A ray that passes out of the basophile cap loses suddenly in intensity of staining (Fig. 8).

The spindle is decidedly denser than the surrounding protoplasm; this has been noted by several investigators (cf. Foot and Strobell (p. 221), and McClendon). The same thing is

shown by some of the phenomena of centrifuged eggs; when, for instance, the direction of centrifugal force is at right angles to the axis of the spindle, granules must stream by it. Now if the density of the spindle area be no more than that of the surrounding ground substance and if there be no repellent force from the spindle, such granules would pass through the spindle readily. But on the contrary they are blocked by the spindle, and heap up against it. Fig. 6 illustrates this condition. The accumulation of granules on the distal side of the spindle is fatty in character, at least the granules are blackened by Flemming's fluid which was used for killing, and they are the same in character as those accumulated at the proximal pole. A pathway of such granules runs from the pole of the spindle towards the proximal accumulation, rendering the interpretation certain. We can readily believe, therefore, that the relatively dense spindle moves as a unit through the protoplasm. If, then, it be thrown into the basophile cap, it will naturally tend to maintain its character; many such cases are in fact found. In other cases, the part of the spindle within the basophile cap is partly changed, partly unchanged; but in very many cases the part within the cap is completely changed, so that the staining reaction of the spindle agrees with that of the cap.

Whether we consider the movements of the karyokinetic figure produced within the egg by centrifuging, or whether we consider the results of driving granules into the area of the karyokinetic figure, the general conclusion that the poles of the spindles are centers of force appears to me to be inevitable; no system of antagonistic fibrillæ could behave in such a way. The difficulties that the centrosome hypothesis has to meet are well known, and their discussion does not enter within the scope of this contribution, which is in a sense a by-product of another problem. I shall therefore be satisfied merely to present the evidence and to indicate the direction in which it appears to point.

In the development of the karyokinetic figure, the rays or fibers appear to grow out of the centers up to the time of the mesophase at least, and this must be due to increasing power of the centers. In a magnetic model on the other hand the orientation of particles along the lines of force takes place simultane-

ously by segregation along their entire course (Hartog, '05). Now my experiments give conditions quite similar to magnetic models in this respect, for the reason that the centers displaced at the mesophase are in the condition of maximum force.

The fact that the experiments deal only with this stage of the karyokinetic figure limits the results in ways that I fully realize. But this is more than compensated by the advantage of knowing the precise stage of the karyokinetic figure with which the operations deal. The attempts to obtain comparable results at other stages have been unsatisfactory, because any other stage is in motion and the control eggs vary so much that interpretation of results becomes very uncertain.

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